association for recruitment, are not bimodal (in size) in our study area.

In this general pattern of recruitment, we cannot distinguish between success of settlement of newly metamorphosed urchins and the survival of somewhat larger animals. For example, our failure to detect substantial recruitment in coralline algae or cobble, as has previously been reported by others (*8*), may reflect good settlement in these microhabitats followed by high susceptibility to predation. Although these distinctions may be important, the main criterion for reproductive success is recruitment of adult size classes. The effects of commercial fishing on recruitment of *S. franciscanus* will probably be determined by the number of adults left behind or the number that migrate into an area after fishing takes place. It is not yet known whether harvesting of *S. franciscanus* will shift the ratios of *S. purpuratus* to *S. franciscanus* as might be expected from the different recruitment patterns of these coexisting species.

MIA J. TEGNER
PAUL K. DAYTON
Scripps Institution of Oceanography, La Jolla, California 92093

References and Notes

1. G. Thorson, in *Treatise in Marine Ecology and Paleoecology*, I, *Ecology*, J. W. Hedgpeth, Ed. (Geological Society of America, New York, 1957), p. 461; *Neth. J. Sea Res.* **3**, 267 (1966).
2. S. Kato, *Mar. Fish. Rev.* **34**, 23 (1972); F. R. Bernard and D. C. Miller, *Fish. Res. Board Can. Tech. Rep.* **400**, 1 (1973).
3. R. T. Paine and R. L. Vadas, *Limnol. Oceanogr.* **14**, 710 (1969); W. J. North and J. S. Pearse, *Science* **167**, 209 (1970); P. K. Dayton, *Fish. Bull.* **73**, 230 (1975).
4. D. L. Leighton, in *Biology of Giant Kelp Beds (Macrocystis) in California*, W. J. North, Ed. (Cramer, Lehre, Germany, 1971), p. 421.
5. J. A. Estes and J. F. Palmisano, *Science* **185**, 1058 (1974).
6. P. K. Dayton, *Ecol. Monogr.* **45**, 137 (1975).
7. R. S. Scheltema, *Thalassia Jugosl.* **10** (½), 263 (1974).
8. D. L. Leighton, L. G. Jones, W. J. North, E. K. Andersen, in *Kelp Habitat Improvement Project, Annual Report for 1965–1966*, W. J. North, Ed. (California Institute of Technology, Pasadena, 1966), p. 84; *ibid.* (1973), p. 16; D. Lees, *ibid.* (1971), p. 70.
9. T. A. Ebert, *Ecology* **49**, 1075 (1968).
10. The density of adult *S. franciscanus* had been increased for experimental purposes 7 months before sampling.
11. S. Schroeter, personal communication.
12. R. T. Hinegardner, *Biol. Bull.* **137**, 465 (1969).
13. *Dermasterias imbricata, Mediaster aequalis, Patiria miniata, Henricia leviuscula, Pycnopodia helianthoides, Astrometis sertulifera, Orthasterias koehleri*, and *Pisaster giganteus*.
14. R. R. Winget, thesis, San Diego State College (1968).
15. L. Deysher, personal communication; K. McCleneghan, thesis, University of Southern California (1968).
16. We thank M. Aeder and R. Walker for assistance in the field and laboratory, and B. Bernstein, V. Currie, T. Gerrodette, and J. Rude for being diving buddies. We also thank T. Schultz for logistic support, L. Deysher for identifying the coralline algae, S. Schroeter for discussion, and L. Ball, B. Bernstein, N. Holland, and J. Oliver for reviewing the manuscript. Supported by NOAA grants 04-5-158-20 and 04-6-158-44021 (from the Office of Sea Grant) and NSF grant DES 75-08661.

7 July 1976; revised 6 October 1976

Antischizophrenic Drugs: Chronic Treatment Elevates Dopamine Receptor Binding in Brain

Abstract. *Chronic treatment of rats with the neuroleptic drugs haloperidol, fluphenazine, and reserpine elicits a 20 to 25 percent increase in striatal dopamine receptor binding assayed with [^{3}H]haloperidol. This increase in receptor sites may account for behavioral supersensitivity to dopamine receptor stimulants in such animals and for tardive dyskinesia in patients treated with these drugs.*

Chronic treatment with antischizophrenic neuroleptic drugs produces motor abnormalities that appear related to the dopamine neuronal systems in the brain in both man and animals. Tardive dyskinesia, characterized by abnormal movements of facial muscles and extremities, is a major complication of long-term treatment with neuroleptic drugs (*1*). Lowering the dose or terminating the drugs frequently worsens these symptoms, while increasing the dose may alleviate symptoms. Since a major action of neuroleptics is blockade of dopamine receptors in the brain, speculations have linked tardive dyskinesia with a supersensitivity of dopamine receptors after prolonged blockade by chronic drug administration. This would explain why a reduction of dose worsens symptoms while a dose increase temporarily reverses motor abnormalities. Studies in animal models of tardive dyskinesia support the notion that the increased motor activity reflects supersensitivity of dopamine receptors. Rats or mice treated chronically with neuroleptic drugs display an enhanced sensitivity to the motor stimulant effects of apomorphine, a direct dopamine receptor agonist, after the neuroleptic treatment is terminated (*2–4*). A similar motor supersensitivity to dopamine receptor stimulants is apparent when dopamine synaptic activity is reduced by inhibiting synthesis of dopamine with α-methyltyrosine (*2, 4*), depleting dopamine storage with reserpine (*2, 5*), or making lesions in the nigrostriatal dopamine pathway (*5, 6*).

Dopamine receptors in the brain can be labeled by direct binding of [^{3}H]-haloperidol (*7*). Binding sites occur only in brain regions rich in dopamine synapses, and the relative potencies of numerous neuroleptic drugs for the binding sites parallel their behavioral activities in animals and man (*8*). We now report enhanced dopamine receptor binding of [^{3}H]haloperidol in the corpus striatum of rats treated chronically with neuroleptic drugs.

Binding assays were performed as described (*8*). Homogenates (Brinkmann Polytron) of fresh rat corpus striatum (40 mg per side) in cold tris (hydroxymethyl) aminomethane (tris) buffer were washed twice by centrifugation. The final resuspension (in cold 50 m*M* tris buffer containing 0.1 percent ascorbic acid, 10 μM pargyline, 120 m*M* NaCl, 5 m*M* KCl, 2 m*M* $CaCl_2$, and 1 m*M* $MgCl_2$; final *p*H of 7.1 at 37°C) was warmed to 37°C for 5 minutes and returned to 4°C. Each tube received 1 ml of tissue suspension (8 mg of wet tissue) and contained 0.2 to 6 n*M* [^{3}H]haloperidol (9.6 c/mmole, Janssen Pharmaceutica). Tubes were incubated at 37°C for 10 minutes, and triplicate 0.3-ml portions were rapidly filtered under vacuum through Whatman GF/B filters with two 5-ml rinses of cold buffer. The filters were counted by liquid scintillation spectrometry. Specific binding of [^{3}H]haloperidol, measured as the excess over blank tubes containing 100 μM dopamine in addition to the above incubation mixture, represented about half of the total binding.

Treatment of rats for 3 weeks (Table 1) with the potent butyrophenone neuroleptic haloperidol produces about a 20 percent increase in specific [^{3}H]haloperidol binding ($P < .0005$). Fluphenazine, one of the most potent phenothiazine neuro-

Table 1. Effect of chronic drug treatments on [^{3}H]haloperidol binding in the rat. Rats were injected subcutaneously with haloperidol (Haldol, 0.5 mg/kg), reserpine (Serpasil, 0.25 mg/kg), fluphenazine (Prolixin, 0.5 mg/kg), promethazine (Phenergan, 2.5 mg/kg), or *d*-amphetamine sulfate (5 mg/kg) daily for 3 weeks and killed 5 to 7 days later. Freshly removed corpora striata were assayed for binding with three concentrations of [^{3}H]haloperidol (0.2 to 1.4 n*M*). Results for the three concentrations were averaged for each rat. Data for each treated rat were expressed as the percentage difference in specifically bound radioactivity relative to that in a matched uninjected control rat assayed in parallel. Means and standard errors of the mean are given; probability values were computed by the one-tailed *t*-test; N.S., not significant. Control values for [^{3}H]haloperidol binding are as given for Table 2.

Injected drug	Number of treated animals	Difference relative to control (%)	*P*
Haloperidol	21	19 ± 4	< .0005
Reserpine	10	23 ± 7	< .005
Fluphenazine	6	27 ± 12	< .05
Promethazine	12	3 ± 7	N.S.
Amphetamine	5	−2 ± 4	N.S.

leptics, produces a similar increase in binding after administration for 3 weeks. By contrast, treatment for 3 weeks with a substantially higher dose of the phenothiazine promethazine, which lacks antischizophrenic neuroleptic activity (*9*), fails to significantly enhance [^{3}H]haloperidol binding. Administration of reserpine, an antischizophrenic drug that depletes brain dopamine, also elicits about a 20 percent augmentation of [^{3}H]haloperidol binding. Treatment with the stimulant amphetamine for 3 weeks fails to change receptor binding significantly. In all of these experiments, dopamine receptor binding was assayed 5 to 7 days after termination of drug treatment. This permitted partial elimination of the drugs from the body, as their continued presence might interfere with dopamine receptor binding. The behavioral supersensitivity to apomorphine is demonstrable for at least a week after neuroleptic treatment of rats is terminated (*2*).

To evaluate the dose characteristics and time course of the changes in receptor binding, we compared the effects of two doses of haloperidol, 0.5 and 5.0 mg per kilogram of body weight, given daily for 1 or 3 weeks (Table 2). We measured [^{3}H]haloperidol binding 5, 12, and 17 days after drug treatment was terminated. The augmentation of binding is as great with the lower as with the higher haloperidol dose. Likewise, the increase in binding after 1 week of haloperidol treatment is similar to that after 3 weeks. Twelve days after haloperidol treatment was terminated, the increased binding is less apparent than at 5 days, while at 17 days no increase is detected.

To determine whether the enhanced receptor binding is attributable to an increased number of binding sites or to a change in affinity, we examined tissue samples from individual rats for binding at five concentrations of [^{3}H]haloperidol ranging from 0.2 to 6.0 nM, and analyzed the data by Scatchard plots drawn by the method of least squares. Five days after termination of haloperidol administration (0.5 mg/kg daily for 21 days), the dissociation constant for [^{3}H]haloperidol binding in the corpus striatum does not differ significantly from that for controls (control: K_D = 0.91 ± 0.06 nM, N = 21; haloperidol treatment: K_D = 0.99 ± 0.09 nM, N = 21). By contrast, there is a 20 to 25 percent increase in the total number of binding sites, corresponding to the similar increase observed in receptor binding at low concentrations of [^{3}H]-haloperidol (control: binding = 27.9 ± 1.4 pmole per gram of tissue; haloperidol treatment: binding = 34.4 ± 1.9 pmole/g; $P < .005$, Student's t-test on paired data).

Table 2. Time course and dose dependence of increase in [^{3}H]haloperidol binding after chronic haloperidol treatment. Rats were injected daily for 1 or 3 weeks with haloperidol, 0.5 or 5 mg/kg, as in Table 1, and were killed 5 to 17 days later. Freshly removed striata were assayed for binding with 0.4 and 0.8 nM [^{3}H]haloperidol. Data are expressed as in Table 1. Each result is the mean and standard error of the mean for five pairs of rats, paired in order of assay. Probability values were calculated by the one-tailed t-test. [^{3}H]Haloperidol binding values at 0.4 and 0.8 nM in control striata were equivalent to 5 and 8 pmole per gram of tissue, respectively.

Treatment			[^{3}H]Haloperidol concentration			
			0.4 nM		0.8 nM	
Injection period (weeks)	Daily dose (mg/kg)	Drug-free period (days)	Increase in binding (%)	P	Increase in binding (%)	P
1	0.5	5	19 ± 5	< .01	36 ± 11	< .025
	5.0		27 ± 7	< .01	15 ± 9	N.S.
3	0.5	5	25 ± 2	< .001	24 ± 9	< .05
	5.0		17 ± 6	< .025	31 ± 7	< .005
3	0.5	12	13 ± 14	N.S.	18 ± 6	< .05
3	0.5	17	0 ± 6	N.S.	8 ± 5	N.S.

Both the clinical motor abnormalities seen after prolonged administration of neuroleptics in man and the enhanced response to apomorphine seen after such treatments in rodents suggest that dopamine receptor sites in the corpus striatum may be supersensitive. However, behavioral supersensitivity to dopamine could be produced by a variety of other mechanisms, such as effects on nondopamine neuronal systems, metabolic changes in cells postsynaptic to dopamine neurons, or behavioral conditioning phenomena (*10*).

Our data indicate that the motor changes seen after chronic neuroleptic treatment are associated with an increase in the number of dopamine receptors. This increase in the number of [^{3}H]haloperidol binding sites is consistent with the behavioral supersensitivity to apomorphine in rats treated with neuroleptics on a similar dose schedule. The greater relative enhancement of apomorphine stimulated effects in such rats (*2*) compared to the increased [^{3}H]-haloperidol binding found here suggests that postreceptor components may also be involved in the increased behavioral response. The activity of a dopamine-sensitive adenylate cyclase in the corpus striatum is not altered in mice treated chronically with neuroleptics (*3*). Moreover, the ability of apomorphine to elevate striatal adenosine 3′,5′-monophosphate concentrations in vivo is unaltered in these mice (*3*). This apparent discrepancy between the increased receptor binding—reflecting the recognition sites of the dopamine receptor—and adenylate cyclase activity, accords with other data indicating that receptor binding sites and associated adenylate cyclase may be distinct entities (*8, 11*).

Behavioral supersensitivity to apomorphine is more pronounced after lesions of the nigrostriatal dopamine pathway than after chronic treatment with neuroleptic drugs. We have observed enhancement of dopamine receptor binding in the corpus striatum of rats with nigrostriatal lesions (*12*). The augmentation of binding in these animals is about twice that observed in rats treated chronically with neuroleptics.

DAVID R. BURT*
IAN CREESE, SOLOMON H. SNYDER
Department of Pharmacology, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

References and Notes

1. G. E. Crane, *Science* **181**, 124 (1973); H. L. Klawans, Jr., *Am. J. Psychiatry* **130**, 82 (1973); G. W. Paulson, *Annu. Rev. Med.* **26**, 75 (1975).
2. D. Tarsy and R. J. Baldessarini, *Neuropharmacology* **13**, 927 (1974); A. V. Christensen, B. Fjalland, J. Møller-Nielsen, *Psychopharmacology* **48**, 1 (1976); H. L. Klawans and R. Rubovitz, *J. Neural Transm.* **33**, 235 (1972); A. C. Sayers, H. R. Bürki, W. Ruch, H. Asper, *Psychopharmacologia* **41**, 97 (1975).
3. P. F. Von Voigtlander, E. G. Losey, H. J. Trienzenberg, *J. Pharmacol. Exp. Ther.* **193**, 88 (1975).
4. K. E. Moore and J. E. Thornburg, *Adv. Neurol.* **9**, 93 (1975).
5. U. Ungerstedt, T. Ljungberg, B. Hoffer, G. Siggins, *ibid.*, p. 57.
6. U. Ungerstedt, *Acta Physiol. Scand. Suppl.* **367**, 69 (1971); S. D. Iversen and I. Creese, *Adv. Neurol.* **9**, 81 (1975).
7. I. Creese, D. R. Burt, S. H. Snyder, *Life Sci.* **17**, 993 (1975); P. Seeman, M. Chau-Wong, J. Tedesco, K. Wong, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 4376 (1975); P. Seeman, T. Lee, M. Chau-Wong, K. Wong, *Nature (London)* **261**, 717 (1976).
8. I. Creese, D. R. Burt, S. H. Snyder, *Life Sci.* **17**, 1715 (1975); S. H. Snyder, I. Creese, D. R. Burt, *Psychopharmacol. Commun.* **1**, 663 (1975); S. H. Snyder, D. R. Burt, I. Creese, *Neurosci. Symp.* **1**, 28 (1976); I. Creese, D. R. Burt, S. H. Snyder, *Science* **192**, 481 (1976); S. H. Snyder, I. Creese, D. R. Burt, in *Neuroregulators and Psychiatric Disorders*, E. Usdin, D. Hamburg, J. Barchas, Eds. (Oxford Univ. Press, New York, in press); D. R. Burt, I. Creese, S. H. Snyder, *Mol. Pharmacol.* **12**, 800 (1976); *ibid.*, p. 631; I. Creese, D. R. Burt, S. H. Snyder, *Science* **194**, 546 (1976).
9. D. F. Klein and J. M. Davis, *Diagnosis and Drug Treatment of Psychiatric Disorders* (Williams & Wilkins, Baltimore, 1969).
10. I. Creese and S. D. Iversen, in *Pre- and Postsynaptic Receptors*, E. Usdin and W. E. Bunney,

Jr., Eds. (Dekker, New York, 1975), pp. 171–190; B. J. Sahakian and T. W. Robbins, *Psychopharmacologia* **45**, 115 (1975).
11. P. Cuatrecasas and M. Hollenberg, *Adv. Protein Chem.* **30**, 251 (1976); P. A. Insel, M. E. Maguire, A. G. Gilman, H. R. Bourne, P. Coffino, K. L. Melmon, *Mol. Pharmacol.* **12**, 1062 (1976); M. E. Charness, D. B. Bylund, B. S. Beckman, M. D. Hollenberg, S. H. Snyder, *Life Sci.* **19**, 243 (1976).
12. I. Creese, D. R. Burt, S. H. Snyder, in preparation.
13. We thank J. Ryan, C. Kaufmann, G. Mack for technical assistance and Janssen Pharmaceutica for the generous gift of [^{3}H]haloperidol. Supported by PHS grant MH-18501, NIMH research scientist development award MH-33128 to S.H.S., PHS fellowships NS-01654 to D.R.B. and DA-05328 to I.C., and grants from the John H. Hartford Foundation and the Scottish Rite Foundation.
* Present address: Department of Pharmacology, University of Maryland School of Medicine, Baltimore, Maryland 21201.

16 July 1976; revised 16 September 1976

Antigen-Antibody Reactions in Rat Brain Sites Induce Transient Changes in Drinking Behavior

Abstract. *The septum or hypothalamus of rat brain was injected through implanted cannulas with antibody against membrane antigens in the rat brain or with antibody against exogenous soluble antigens (such as ovalbumin) followed by the specific antigen. Both immunological systems produced a moderate but highly significant decrease in drinking by thirsty rats. This phenomenon is suggested as an experimental model for behavioral disorders resulting from nondegenerative, immunological processes in the brain.*

Intracranial injection of antiserum against brain has been employed since 1900 (*1*) to explore possible roles of immunological reactions in neural dysfunction and behavioral disorders. More recent investigation of immunological reactions in the brain has included the search for specific brain antigens and their localization (*2, 3*), the behavioral effects (*4, 5*) and the electrophysiological effects (*4, 6*) of antibody against brain, and the behavioral effects of active immunization of animals with brain antigens (*7*).

The objective of our study has been to elucidate the mechanisms by which immunological reactions in the brain affect behavior. Three broad categories of immunological processes are considered. One is neural cell damage by activated cytolytic pathways such as complement or by degenerative inflammatory lesions (*8*). Another is direct modification of neural cell function, either stimulation by cell surface "activation" or inhibition by blockade of neurochemical receptor sites. The third immunological process proposed is noncytotoxic release of pharmacologically active substances that affect neurotransmission (*9*).

Two types of immune reaction systems were employed. One was rabbit antibody against rat brain tissue antigens. The other was an immunochemically defined and quantifiable reaction with rabbit antibody and a crystalline antigen unrelated to brain. The antiserums against brain are operationally defined since they could be expected to have a variety of antibody specificities including some for generalized rat tissue antigens, for serum proteins, and perhaps for the adjuvant employed. The control reaction systems consisted of hen egg ovalbumin (OA) and human serum albumin (HSA) and their respective rabbit antiserums.

If a behavioral effect is found with antibody against brain, the simplest assumption is that antibody has combined with a substance on a cell surface and has thereby initiated cellular damage or has otherwise modified normal cell function. Determining the effect of a defined reaction system with an antigen not found in the rat brain provides either a control for the rabbit immunoglobulin preparation or a demonstration that an antibody-antigen reaction in the brain can indirectly alter neural function.

We have chosen a relatively simple behavioral response system—drinking by water-deprived rats—to assess the effects of the immunological reaction. By contrast, all previous experiments conducted for this purpose have demonstrated impaired performance of learned or conditioned responses. A change in any behavior can serve to detect an antibody effect and, with conventional controls, perhaps demonstrate the specificity of the antibody for brain antigens. The investigation of mechanisms, however, depends upon a predictable relationship between behavior and its physical substrate processes. We chose the drinking response because much is known about the neural substrates and the neurochemical modulation of water intake (*10*). The use of this simple indicator of neural function allows us to relate the changes in response patterns induced by immunological reactions to regulatory processes and pathways already established by neuroanatomical localization studies using electrical and chemical stimulation techniques and lesions.

The experiments reported here demonstrate that the completely exogenous immune reaction induces a behavioral effect similar to that of the antibody against brain. The results and the analysis of our data in the light of reported effects of lesions, electrical stimulation, and chemical treatments lead us to prefer noncytotoxic release over the cell damage or receptor blockade models.

Antiserums prepared in rabbits against immunogenic fractions of rat brain and control antiserums against HSA and OA were processed to give immunoglobulin concentrates (*11*). Preparations were injected into the rat brain through cannulas implanted in septal or perifornical hypothalamic sites (*12*) involved in the regulation of drinking behavior (*13, 14*).

Charles River CD male rats (250 to 300 g) with intracranial cannulas were adapted to a 23-hour water deprivation schedule. This schedule continued for at least 10 days when each animal's daily water intake had stabilized at a characteristic level within a group range of 20 to 30 ml/day. The 1-hour daily drinking period began at 4 p.m., 2 hours before the onset of the dark period of the light-dark cycle. Purina Lab Chow was continuously available and animals were handled daily before the drinking period. Tests were separated by at least 1 week to allow animals affected by the previous experiment to reestablish normal baseline drinking. The effect of the test substance was assessed by comparing intake on day −1, a baseline measure, with that of day 0, on which the test substance was injected, and days 1 and 2, during which the course of recovery to baseline behavior was charted.

Screening rests were performed to identify active antiserums. Preparations of immunoglobulin from rabbits immunized against various brain fractions were administered in the septal site in two 2.5-μl injections with a 20- to 30-minute interval; the second injection immediately preceded the afternoon drinking period. Depression of drinking was observed following treatment with antiserums against a septal microsomal fraction, against a cerebellar microsomal fraction, and against a cortical microsomal fraction. Two antiserums against soluble fractions of septal and cerebellar homogenates, respectively, had several antibodies distinguishable by immunoelectrophoresis but, when tested in the same animals, produced minimal or no behavioral responses. The antiserum having the most pronounced effect was one against a septal microsomal fraction. The immunoglobulin from this antiserum was used in the experiments reported below as a representative antibody against brain and has been designated antibody against rat brain membrane (aRBM).